In the Specification:

Please amend the paragraph at page 2, lines 7-18 as follows:

The plaques and neurofibrils described by Alzheimer, which are today called senile plaques and neurofibrillary tangles (NFT), are used as a definitive diagnosis of AD (Figure 1A (Palmert, M.R. *et al.* (1996) *Science* 24:11080-11084). The plaques and tangles are seen primarily in the hippocampus, amygdale, and the cerebral cortex (Van Broeckhoven, C.L. (1995) *Eur. Neurol.* 35:8-19). Evidence for either a molecular or immunological disease origin may be found in the plaques and tangles, depending upon a researcher's point of reference. From a molecular perspective, the initial identification of specific mutations within the amyloid precursor protein (APP) (Schellenberg, G.D. *et al.* (1991) 49:511-517) and the presence of Aβ (a derivative of APP) in plaques points to a unique protein cause for AD.

Please amend the paragraph bridging pages 4 to 5 as follows:

Besides plaques, the intracellular neurofibrillary tangles are often also characteristic of AD brain tissue. The plaques and neurofibrillary tangles are the primary diagnostic features of AD. However, immunohistochemical staining is becoming more useful as additional antibodies specific to plaque components are developed. An immunodominant region of APP has been localized to the C-terminal tail. This region of the processed APP had been postulated to remain intracellular and was recently shown to accumulate specifically in the neuronal cells of the hippocampus and amygdale of AD patients, but not in similar tissue of the normal age-matched patients (Kotwal, G.J. *et al.* (1997) *Soc. Neurosci. Abstr.* 22:502). As shown in ~~the right portion of~~ Figure 1B, a high titer antibody to the immunodominant region can give rise to specific intracellular immunohistochemical staining in the amygdale, which may someday find routine usage to confirm diagnosis in conjunction with clinical history and silver staining. Additionally, the presence of the C-terminus demonstrates that the C-terminal tail of APP accumulates intracellularly in neural tissue of those suffering from AD.

Please amend the text at page 16, lines 11-22 as follows:

M K [[E V]] V E S V T F L T L L G I G C V L S C C T I P S

R P I N M K F K N S V E T D A N A N Y N I G D T I

E Y L C L P G Y R K Q K M G P I Y A K C T G T G W
T L F N Q C I K R R C P S P R D I D N G Q L D I G
G V D F G S S I T Y S C N S G Y H L I G E S K S Y
C E L G S T G S M V W N P E A P I C E S V K C Q S
P P S I S N G R H N G Y E D F Y T D G S V V T Y S
C N S G Y S L I G N S G V L C S G G E W S D P P T
C Q I V K C P H P T I S N G Y L S S G F K R S Y S
Y N D N V D F K C K Y G Y K L S G S S S S T C S P
G N T W K P E L P K C V R
(SEQ ID NO:1)

Please amend the Brief Description of the Drawings section from page 17, line 5 through page 24, line 14 as shown below:

Figure 1. AD Brain Tissue. Figure 1A ~~The left photograph~~ shows a conventional silver stained section of brain tissue from postmortem AD patient showing the characteristic hallmark of AD, the amyloid/neuritic plaques (indicated by arrows). The central portion of a plaque is a dense, dark brown to black region surrounded by a clear ring and then a ring of particulate matter comprising cell debris, activated microglial cells, and several different proteins. Figure 1B ~~The right photograph~~ shows immunohistochemical staining of a microsection of the amygdala (a brain structure that plays a major role in emotions such as fear and anxiety and in the startle response) from a postmortem AD patient. A high-titer antibody raised in a rabbit against a synthetic peptide whose sequence was derived from a computer–generated immunodominant region within the putative intracellularly localized C-terminal tail of APP was used as a primary antibody. This was followed by a peroxidase-conjugated anti-rabbit secondary antibody. Brown color developed on addition of DAB reagent. The nuclei were finally stained with hematoxylin. The [[the]] numerous brown threads seen specifically in the AD tissue are indicative of the presence of the cytoplasmic C-terminal tail of APP in the neuropils (dead/dying neurons).

Figure 2. A detailed diagram of the major isoform of amyloid precursor protein (APP_{695}) present in neurons. The 17-amino acid signal peptide is cotranslationally cleaved in the endoplasmic

reticulum. The APP has a cysteine-rich region (8 cysteines within a span of 150 amino acids), as well as an acidic region with a high percentage of the acidic amino acids, glutamic acid and aspartic acid. A threonine-rich region is marked Thr. Alternatively spliced mRNA (in tissues elsewhere in the body) may have an insertion of up to 225 nucleotides that encodes a 56-amino acid Kunitz protease inhibitor region (KPI) to form the isoform APP_{751}, and another additional 19-amino acid region (isoform APP_{770}). The APP anchors to the cell surface via a membrane-spanning region. Depending on the type of proteolytic cleavage, the cell surface APP can either be digested by α-secretase and gamma-secretase to give rise to α-APPs (584 amino acids) and a small P3 protein, which are soluble and are released into the extracellular space, with the C-terminus staying behind (as shown in the middle diagram). Alternatively, it may be digested by β-secretase and gamma-secretase to give rise to Aβ containing a region (40-42 amino acids) that is secreted along with β-APPs (578 amino acids), with the C-terminal tail remaining cell associated. The Aβ aggregates to form the nucleus of the amyloid plaque and has a region that can bind C1q and activate the classical complement pathway. The Aβ can also couple to C3 via an ester linkage and activate the alternative pathway. The cytoplasmic C-terminal tail has been speculated to trigger a cascade of events contributing to the apoptosis of the APP-producing neurons. Such a cascade is thought to be initiated by the binding of the C-terminal region to a particular family of GTP binding proteins (G_0).

Figure 3. The aberrant biosynthesis of APP accounts for some of the amyloid plaque formation. This is complicated by other proteins regulating the amount of APP synthesized and process, such as the presenillins 1 and 2. The [[the]] neurofibrillary tangles (NFTs) may be formed when [[with]] the tau protein homodimerizes in the presence of the epsilon 4 allele of apoE, statistically found more often in persons susceptible to neurodegenerative disorders. The second and third alleles may be able to block the sites on tau that contribute to the formation of homodimers, thereby contributing to the normal cytoskeletal structure. ApoE, besides having sites for binding to its receptor, has a lipid binding site to which Aβ can bind. Aβ can thus be transported from astrocytes to the neuronal surface and recycled via endosomes and lysosomes to remain cytoplasmic and become incorporated in the neurofilbrillary tangles.

Figure 4. Antibody-independent activation of the classical complement pathway (CCP) and initiation of inflammatory events in AD. Aβ, its aggregate, or the amyloid plaque can trigger the CCP by binding to C1q~~, shown in blue~~. The binding to C1q occurs via the first few amino acids of Aβ (4-11), with the aspartate in position 7 being the most critical for binding. Aβ or its aggregate can form ester links to the C3 component and can activate the alternate complement pathway (ACP)~~, shown in yellow~~. Complement activation can result in the release of chemotactic factors such as C3a, C4a, and C5a~~, shown here in green~~. These factors can cause an influx of microglial cells and astrocytes, which contribute to the inflammatory response. These cells are also present in the neuritic plaques.

Figure 5. Generation of recombinant plasmid pAPPc. PCR was performed on a modified pUC 18 vector, pFB68L, containing the APP_{695} C-terminus (nucleotides 1786 [[I786]]-3207) incorporated at the *Eco*RI restriction site. The primers were designed so that the [[1.I]] 1.1 kbp PCR product would contain the two new restriction sites *Nco*I and *Sal*I. Digestion of the PCR product and pTM3 with *Nco*I and *Sal*I and subsequent ligation produced plasmid pAPPc. Abbreviations: multiple cloning site (MCS), T7 bacteriophage promoter (pT7), termination sequence (T7t), encephalomyocarditis virus "translational enhancer" leader sequence (EMC), guanosyl phophoribosyl transferase (GPT), 7.5 kbp vaccinia promoter (p7.5K), thymidine kinase (TK), origin of replication (ori), ampicillin resistance gene (ampr).

Figure 6 shows the purification of pAPPc. Figure 6A shows a purified and restricted clone of pAPPc electrophoresed through an agarose gel. Figure 6B shows twelve purified and restricted clones of pAPPc electrophoresed through an agarose gel. Figure 6C shows a Southern blot of the twelve pAPPc clones from the agarose gel shown in Figure 6B.

Figure 7 [[6]]. Sequence of pAPPc within insert and flanking regions. The DNA (SEQ ID NO:3) and amino acid (SEQ ID NO:4) sequences of pAPPc and the flanking pTM3 sequence is shown with primer sets, restriction sites and important regions noted. The[[.]] arrows indicate the location and direction of the five sets of primers synthesized for DNA sequencing.

Figure 8 [[7]]. Flow chart of distribution of mice into groups. Thirty-two animals were injected in a series of three experiments with [[ei-ther]] either Tris-buffered saline or Tris buffer, control product (pTM3) or C-100 (APP), or AB peptide. The mice were sacrificed at either five (5h) or 48 (48h) hours after injection. C5+/+ and C5-/- mice were used. Numbers in parenthesis indicate the number of individual mice in each group.

Figure 9 [[8]]. Connective tissue Air pouch cell counts. Cell count bars are means of all gfids counted from animals injected with 100 microliters of sample listed on x-axis, plus or minus standard deviation shown as an error bar. Figure 9A [[4A]] is a summary of counts from mice sacrificed five h post injection. Figure 9B [[4B]] is a summary of counts from mice sacrificed 48 h post injection. Abbreviations: C5 sufficient (C5+/+) or deficient (C5-/-) mice, male (M), female (F), control product (pTM3) or C-100 [[C-I00]] (pAPPc), AB peptide (AB), eosinophil (Eosin), neutrophil (Neutr), monocyte (Mono).

Figure 10 [[9]]. Air pouch connective tissue section composite. Representative photos of single-layer connective tissue spreads from one experiment. Left column panels (Figures 10 [[5]]A, C, E and G) are from C[[-]]5-/- mice injected, whereas right column panels (Figures 10B [[58]], D, F and H) are from C5+/+ mice injected. Panels 10 [[5]]A, B, C and D are from mice sacrificed five hours after injection. Panels 10 [[5]]E, F, G and H are from mice sacrificed 48 h after infection. Mouse sections in panels 10 [[5]]A, B, E and F were from animals injected with *in vitro* transcription/translation product encoded by control pTM3 DNA, and those in panels 10 [[5]]C, D, G and H were injected with product from *in vitro* transcription/translation reaction encoded by pAPPc DNA. Migrating cells found most frequently in epithelial air pouch sections are indicated: resident fibroblast, (←) neutrophil, (■) eosinophil, (♦) monocytes. The magnification used is [[in]] 400X.

Figure 11 [[10]]. Immunoassay for complement activation by Aβ. Figure 11A [[6A]]. Internal controls for quantitation of results in ng/mi included standards A, B and C; and high (HPC) and low (LPC) positive controls. Also, external controls of heat activated IgG (HAG) and zymosan

were included for confirmation of intact complement pathway in normal (NHS) and agammaglubulinemic (AHS) human sera. Figure 11B [[6B]]. Measurement of NHS complement activation in the presence or absence of 50 μM Aβ peptide (A-beta(50)). Inhibition of complement activation by Aβ [[AB]] in NHS was determined by the addition of purified VCP. Background activation is demonstrated by the incubation of NHS with TBS. Standard deviation is indicated by error bars. Figure 11C [[6C]]. Measurement of AHS complement activation in the presence or absence of 50 μM Aβ peptide, with or without added VCP. Background activation is demonstrated by the incubation of NHS with TBS. The addition of MEM to all reactions was necessary because VCP is solubilized in it and also MEM seems to enhance complement activation by Aβ.

~~Figure 11. Intervention by VCP into proposed inflammatory cascade due to Aβ complement activation. Aggregated Aβ activates the complement cascade that results in the deposit of complement components in the nascent plaque as well as releasing chemotactic factors. These factors such as C5a can stimulate an immune response that results in the activation and influx of microglial cells. The accumulated microglia cause more local tissue damage and amplify and perpetuate the inflammation, which results in plaque growth. VCP inhibits both pathways of complement activation by binding to complement components C3 and C4, which results in the diminished preferential release of the chemotactic factor C5a, as demonstrated by the X superimposed over the arrow. Therapeutically, this could alleviate some of the tissue damage caused by a self-perpetuating inflammatory response.~~

Figure 12. Representative histograms showing the VCP is able to reduce mouse antihuman HLA class I antibody binding to HUVECs cells. The results of flow microflourimetric analysis are as follows: Panel 12A) Nonspecific mouse monoclonal antibody (isotype-matched mouse IgG2a mAb) binding to HUVECs cells (negative control); Panel 12B) Mouse monoclonal antihuman HLA-ABC antibody binding to HUVECs cells (positive control); Panel 12C) Mouse monoclonal antihuman HLA-ABC antibody binding to HUVECs cells in the presence of 5 μl (2 μg) of VCP; Panel 12D) Mouse antihuman HLA-ABC antibody to HUVECs cells in the present of 20 μl (5 μg) of VCP.

Figure 13. PAGE analysis of the heparin binding activity of BSA, HBP, lysozyme, and MIP-1 alpha. In order to calibrate the HiTRAP heparin column, various proteins (BSA, HBP, lysozyme, and MIP-1α) with differing affinities for heparin were passed through the column and eluted with sodium chloride concentrations ranging from 250 mM to 4.0 M. M = molecular weight marker, S.M. = starting material, Unb. = unbound fraction, W = wash.

Figure 14. PAGE analysis of the heparin binding activity of VCP and rVCPs. VCP and various rVCPs were passed through separate HiTRAP heparin columns and eluted with sodium chloride concentrations ranging from 250 mM to 2.5 M. The fractions were collected, run on SDS PAGE, silver stained, and the band densities measured. The results are shown as follows: Panel 14A) recombinant rVCP SCR (1,2); Panel 14B) recombinant rVCP SCR (2,3); Panel 14C) recombinant rVCP SCR (3,4); Panel 14D) recombinant rVCP; Panel 14E) VCP from the natural infection process. For Panels [[gels]] A-E, M = molecular weight marker, S.M. = starting material, Unb. = unbound fraction, W = wash, VCP = VCP from natural infection. Panel 14F) Densitometric scanning of Panels ~~above gels~~ 14A-E.

Figure 15. Sequence alignment including termini of rVCP constructs and putative heparin binding sites. Multiple alignment of the four short consensus repeats (SCR) from orthopoxviruses VAC-COP (vaccinia virus, copenhagen strain; SEQ ID NO:5) (Goebel et al., 1990 *Virology* 179:247-263), VAC-WR (vaccinia virus, western reserve strain; SEQ ID NO:6) (Kotwal, G.J. et al. 1989 *Virology* 171:579-587), CPV-GRI (cowpox virus, Russian isolate from human patient; SEQ ID NO:7) (Schelkunov, S.N., et al. 1998 *Virology* 243:432-460), CPV BRI (cowpox virus, Brighton strain; SEQ ID NO:8) (Miller, C.G., et al. 1995 *Cell Immunol.* 162:326-332), VAR-BSH (variola virus, Bangladesh strain; SEQ ID NO:9) (Massung, R.F., et al. 1994. *Virology* 201:215-240), VAR-IND (variola major virus, Indian strain; SEQ ID NO:10) (Schelkunov, S.N., et al. 1998 *Virology* 243:432-460), VAR-GAR (variola minor virus, alastrim Garcia strain; SEQ ID NO:11) (Massung, R.F., et al. 1996. *Virology* 221:291-300), and MPV-ZAI (monkeypox virus, isolated from a human patient from Zaire in 1996; SEQ ID NO:12). The

putative heparin binding sites (K/R-X-K/R) are marked with solid bars, arrows indicate the termini of the rVCP constructs, and the cysteines are highlighted.

Figure 16. Structure-function summary table of VCP, VCP homologs, and rVCPs. VCP/IMP/SPICE, MPV homology of VCP, recombinant VCP, and four recombinant segments of VCP are shown above along with whether they are able to inhibit hemolysis of sensitized sheep red blood cells and/or bind heparin (IMP = inflammation modulatory protein) Also listed are the number of positively charged amino acids (K+R) found in the protein, percentage of positively charged amino acids (%K+R) making up the protein, pI of the protein, and number of putative heparin binding sites found on the surface of the protein.

Figure 17. VCP model showing the heparin binding sites. Front (Figure 17A) and back (Figure 17B) views of the modeled structure of VCP SCR (1-4) showing the heparin binding sites ~~(differentially colored)~~. In order to differentiate the extents of the individual modules, they are shaded appropriately.

Please amend the text bridging pages 24 to 25 as follows:

M K [[E V]] V E S V T F L T L L G I G C V L S C C T I P S
R P I N M K F K N S V E T D A N A N Y N I G D T I
E Y L C L P G Y R K Q K M G P I Y A K C T G T G W
T L F N Q C I K R R C P S P R D I D N G Q L D I G
G V D F G S S I T Y S C N S G Y H L I G E S K S Y
C E L G S T G S M V W N P E A P I C E S V K C Q S
P P S I S N G R H N G Y E D F Y T D G S V V T Y S
C N S G Y S L I G N S G V L C S G G E W S D P P T
C Q I V K C P H P T I S N G Y L S S G F K R S Y S
Y N D N V D F K C K Y G Y K L S G S S S S T C S P
G N T W K P E L P K C V R
(SEQ ID NO:1)

Please amend the text bridging pages 28 to 29 as follows:

M K [[E V]] V E S V T F L T L L G I G C V L S C C T I P S
R P I N M K F K N S V E T D A N A N Y N I G D T I
E Y L C L P G Y R K Q K M G P I Y A K C T G T G W
T L F N Q C I K R R C P S P R D I D N G Q L D I G
G V D F G S S I T Y S C N S G Y H L I G E S K S Y
C E L G S T G S M V W N P E A P I C E S V K C Q S
P P S I S N G R H N G Y E D F Y T D G S V V T Y S
C N S G Y S L I G N S G V L C S G G E W S D P P T
C Q I V K C P H P T I S N G Y L S S G F K R S Y S
Y N D N V D F K C K Y G Y K L S G S S S S T C S P
G N T W K P E L P K C V R
(SEQ ID NO:1)

Please amend the paragraph at page 47, lines 5-21 as follows:

The new recombinant plasmid, pAPPc, was transformed into competent *E. coli* JM105 (Gibco BRL) and transformants were selected for their ability to form colonies on media containing ampicillin. Colonies were selected and further amplified before isolation of the plasmid DNA by alkaline lysis minipreparation (Maniatis, T. et al. (1984) *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Press, New York, p. 363). A clone transformed with pAPPc, as determined by restriction analysis, was amplified and plated on selective media to analyze for purity (Figure 6A). Twelve colonies were chosen and the plasmid DNA amplified and digested as before and checked for homogeneity on an agarose gel (Figure 6B) and by Southern hybridization (Figure 6C) (Southern, E.M. (1975) *J. Mol. Biol.* 98:503-517). The correct sequence of the inserted DNA was confirmed by the dideoxy chain-terminating method (Sanger, F. *et al.*, (1977) *Proc. Natl. Acad. Sci. USA* 74:5463-5467) using the Fidelity DNA Sequencing System manufactured by Oncor (Gaithersburg, MD) (Figure 7 [[6]]). Five sets of primers from within the known sequence of APP were utilized for this sequencing (arrows in Figure 7 [[6]]).

Please amend the paragraph bridging pages 48 to 49 as follows:

The mice were physically immobilized, and 1 ml of air was injected into the skin (subcutaneously) of the animal's dorsum to form a connective tissue air pouch. 100 µl of blinded/coded sample of Tris buffer was then injected into the nascent air pouch of an identical strain mouse. The animals were then sacrificed at different time intervals by an overdose of anesthesia. Figure [[7]] 8 is a flowchart demonstrating the distribution of animals for injection with various samples and the time points after injection of sacrifice. The time points were selected on the basis of expected times when an *in vivo* reaction would occur.

Please amend the paragraph at page 51, lines 14-29 as follows:

As described in Figure 5, PCR amplification of pFB68L containing DNA coding for the C-terminal 100 amino acids of APP with primers JD01F and JD01R resuled in a cDNA with newly engineered *N*col and *Sal*l restriction endonuclease sites at its 5' and 3' ends, respectively. A calculated size of 1.1 kbp was confirmed by agarose gel electrophoresis. After digestion with *N*col and *Sal*l, the cDNA and plasmid pTM3 was transformed into competent *E. coli*, amplified, and purified using a maxiprep kit (Qiagen, Chatsworth, CA). The pAPPc was sequenced and the precise open reading frame expressed along with the primers used for sequencing is shown in Fig. [[6]] 7. The pAPPc was then used in an *in vitro* wheat germ extract coupled transcription/translation reaction. A product of approximately 10 kDa termed C-100 was observed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and its identity was confirmed as corresponding to the APP C-terminus by immunoprecipitation with antibodies raised against the carboxyl terminus of APP.

Please amend the paragraph at page 52, lines 2-13 as follows:

Based on encouraging preliminary observations suggesting cellular influx only in the presence of Aβ in a pilot study with eight mice, 32 additional mice were employed to evaluate the effect of Aβ, with the experimenter blinded to the samples being investigated. The number of mice injected in this study and the distribution of animals for injection with different samples are shown in Figure [[7]] 8. Thirty-two animals were injected over three different experimental runs with either TBS or Tris as background controls, products from *in vitro* coupled

transcription/translation reactions containing either pTM3 or pAPPc DNA, or fibrillar Aβ peptide. Samples were injected into air pouches in either C5+/+ or C5-/- mice. After injection, an incubation period of either five or 48 h was allowed before the animals were sacrificed and tissues were taken.

Please amend the paragraphs bridging pages 52 to 53 and 53 to 54 as follows:

Figure [[8]] 9 is a compilation of the results in graphical form, and represents the averages of the different experimental variations. At 5 h post-injection (Figure [[8]] 9A), the numbers of monocytes and eosinophils present in the different spreads were nearly the same. The neutrophil counts, however, showed a significant increase in connective tissue excised from C5+/+ mice injected with C-100. Since neutrophil numbers were not increased in mice injected with *in vitro* reaction from the control pTM3 vector DNA, it must be the specific C-100 produced only in the *in vitro* reactions that is stimulating neutrophil influx into the region, and not the reaction mix in general. The possibility that the Aβ fibril formation is enhanced by the coupled transcription-translation reaction cannot be ruled out. Also, no notable neutrophil increase is seen in any of the injected C5-/- mice. This indicates that the inflammatory response present in a few of the mice must be complement-mediated since elimination of a critical fifth component (C5) of the cascade also diminishes cellular influx. Overall, this result seems to be indicative of an early inflammatory response in the presence of complement. In order to determine if the means of the cell count of the C5+/+ mice injected with C-100 or with the control were significantly different, the Mann-Whitney Rank Sum test was performed. The difference in the median values of the 2 groups was greater than would be expected by chance-, there was a statistically significant difference ($P<0.001$). Similar results were obtained when an unpaired t-test was used. There also seemed to be a significant difference between the male and female mice injected with C-100. At 48 h after injection, monocyte numbers were increased, compared to 5 h (Figure [[8]] 9B), whereas neutrophil levels had returned to control levels. The average number of monocytes seen in sections injected with C-100 or Aβ peptide were higher than those seen in tissues injected with either Tris/TBS or pTM3 control. Also, monocyte numbers in C5+/+ mice injected with pAPPc-encoded product or Aβ peptide injected C5+/+ mice were slightly higher than in their C5-/- counterparts.

A general change in the number and type of cells was observed, as is apparent in the composite photo, showing representative fields, from the different experimental situations (Figure [[9]] 10). The early response (Figure [[9]] 10D) showed an influx of neutrophils to the site of injection when the material injected into C5+/+ was the APP C-terminus. Later, at 48 h, post-injection (Figure [[9]] 10H), there seemed to be an increase in the number of mononuclear cells present in experimental sections, but not in controls. At later time points, the number of neutrophils was markedly decreased. Other cell type numbers remained relatively unchanged regardless of what was injected, or the amount of time allowed to pass before the sacrifice of the animal.

Please amend the three paragraphs at page 54, lines 5-29 as follows:

Addition of 50 μM Aβ to twenty-fold diluted NHS resulted in strong activiation of the complement cascade, as measured by formation of SC5b-9 in an enzyme immunoassay. The quantity of activation was extrapolated from control standards with their own internal controls (Figure [[10]] 11A) and expressed in ng/ml. External controls of zymosan and heat-activated IgG (HAG) were also used to confirm complement activation by intact alternative and classical pathways, respectively. These external controls exhibited very high levels of activation (Figure [[10]] 11A), beyond the measurement of the plate reader under experimental conditions.

When NHS was incubated at 7°C for 90 min in the presence of 50 μM Aβ, high levels of complement activation were detected in the range of 150-200 ng/ml as compared to insignificant levels in the absence of Aβ (Figure [[10]] 11A). Addition of purified VCP to the reaction containing NHS and Aβ consistently resulted in complete inhibition (90% or 9-fold) of complement activation to background levels (Figure [[10]] 11B).

When these experiments were repeated, substituting agammaglobulinemic serum for NHS, with an intact complement protein pathway as determined by hemolysis assay (see methods) for NHS, levels of activation very similar to those seen using NHS were observed (Figure [[10]] 11C). Addition of VCP to samples incubated with AHS and Aβ again resulted in inhibition of activation. Serum from agammaglobulinemic patients is devoid of IgM and deficient in IgG, but allowed complement activation similar to the serum with normal levels of

IgG and IgM. This confirmed earlier observations that activation of complement by Aβ is independent of either IgG or IgM anti-body.

Please amend the paragraph bridging pages 55 and 56 as follows:

Histopathological evidence of AD coupled with *in vitro* studies showing that Aβ fibrils can cause complement activation via the classical complement pathway point to an immune response as having an important role in disease progression (Cribbs, D.H. *et al.* (1997) *Neuroreport.* 8:3457-3462, Jiang, H. *et al.* (1994) *J. Immunol.* 152:5050-5059; Mann, D.M.A. *et al.* (1995) *Acta Neuropathol* 90: 472-477; Webster, S. *et al.* (1997). *J. Neurochem.* 69:388-398). The presence of activated microglia, reactive astrocytes, acute phase proteins and complement factors within and around plaques are all signs of an inflammatory response. It is known that the amyloid deposition is capable of causing activation of the classical pathway of the complement cascade in an antibody-independent manner. The complement system forms one of the first molecular lines of defense against infectious agents. Uncontrolled, it can trigger autoimmune destruction of healthy tissue. Deposition of complexes and formation of immunomodulators by the cascade have been associated with activating microglia (Elkabes, S. *et al.* (1996): *J. Neurosci.* 16:2508-2521), the macrophages of the brain, which in turn cause a progression and maintenance of the inflammation, by secreting cytokines like IL-I which would attract other immune cells and astrocytes ~~(Fig. 11)~~. Local tissue destruction follows, along with a further persistence of a chronic form of inflammation. In direct suggestion for the pivotal role of inflammation in AD is its reduced incidence in patients routinely receiving nonsteroidal anti-inflammatory agents to reduce inflammation due to other causes such as arthritis. The use of anti-complement and anti-inflammatory therapies would greatly augment the treatment of AD (Breitner, J.C. *et al.* (1995) *Neurobiology aging* 16(4):523-530; Mackenzie, I.R. & Munoz, D.G. (1998) *Neurology* 50(4):986-990; McGeer, P.L. *et al.* (1996) *Neurology* 47(2):425-432). Prior to the present invention, anti-cholinesterase inhibitors (e.g. tacrine, donepezil), that may also decrease secretion of the amyloid precursor protein, have been the only effective therapy for AD, and only in a limited number of patients.

Please amend the text from page 62, line 24 through page 64, line 24 as follows:

In order to determine the biological consequences of heparin binding by VCP, we tested whether VCP was able to block functional molecular interactions with human vascular endothelial cells. Flow cytometric analysis of HUVECs treated with mouse monoclonal antibodies to human MHC class I molecules - in the presence and absence of VCP – show that VCP is able to modulate antibody binding in a dose-dependent manner. As shown in Figure 12 ~~figure 1~~, addition of 5 µl (2 µg) of VCP to antibody treated HUVECs, reduced antibody binding from 91.5% down to 75.4%. An additional 15 µl (5 µg total) of VCP reduced antibody binding from 91.5% to 61.0% - suggesting that it is blocking in a dose-dependent manner.

To calibrate the HiTRAP heparin column, several proteins having different binding affinities for heparin were passed through the column and eluted with increasing sodium chloride concentrations. SDS PAGE analysis of the eluted fractions containing these proteins of various heparin-binding abilities can be seen in ~~figure 2~~ Figure 13. Analysis suggests that BSA does not bind heparin, since it is present only in the unbound and wash fractions. Lysozyme and MIP-1 α, which bound heparin with moderate and equal affinity, were contained within the 500mM and 750 mM sodium chloride fractions. HBP, showing the highest affinity for heparin, was eluted by a sodium chloride concentration of 2.0 M.

In order to better characterize the heparin binding ability of the VCP molecule, VCP, recombinant VCP, and various recombinant segments of VCP were passed through HiTRAP heparin columns and analyzed using SDS PAGE, shown in ~~figure 3~~ Figure 14. The results suggest that the full-length natural and recombinant proteins bind heparin with close to or equal affinity as lysozyme and MIP-1 α; both VCP and rVCP were concentrated primarily in the 500 mM and 750 mM sodium chloride fractions. PAGE analysis of the various recombinant VCP segments revealed even more interesting data. Recombinant rVCP SCR (1,2) and rVCP SCR (3,4) bound heparin with the same strength as the full-length protein, eluting once again at 500 mM and 750 mM. While recombinant rVCP SCR (2,3), on the other hand, did not bind heparin at all and was found primarily in the unbound and wash fractions. The activity of the purified proteins was then tested using the hemolysis assay. The results indicate that only the full-length protein inhibits lysis of sensitized sheep red blood cells – anywhere from 60 to 90% inhibition. The rVCP segments showed no inhibition of lysis; suggesting that the whole protein is needed to block complement activation. Although, the naturally truncated VCP homolog produced by

monkeypox virus (MPV (shown in ~~figures 4~~ Figures 15 and [[5]] 16), which lacks almost the entire fourth SCR, has been shown to inhibit hemolysis of sensitized sheep red blood cells. Thus, indicating that it is blocking the classical pathway of complement activation.

To more precisely identify the molecular basis behind the VCP-heparin interaction, the amino acid sequences were analyzed using the MacVector software system. Putative heparin binding sites (K/R-X-K/R) were first identified – shown in ~~figure 4~~ Figure 15 along with the sequence alignment of different pox virus VCP homologs – and predicted to be located on the surface of the protein. The amino acid sequences were then scanned to determine the total number of positive amino acids (K+R), the percentage made up of these positively charged amino acids (%K+R), and overall pI of the protein – the results are summarized in ~~figure 4~~ Figure 15. All of the proteins shown to bind heparin had an overall pI of greater than 7.0, and more importantly, were made up of greater than 9% positively charged amino acids. The results also suggest that SCR 1 and SCR 4 contribute the most to this interaction. Although the results show there is no simple answer to how VCP binds heparin, it is clear that the overall positive charge, number of putative binding sites, and the percentage of positive amino acids making up the protein, have a significant effect on its ability to interact with and bind heparin.

Please amend the paragraph at page 66, lines 13-19 as follows:

In order to better visualize the positioning of heparin binding sites in the VCP molecule, a model was developed using four VCP module structures: VCP SCR 3, VCP SCR 4, factor H (fH) SCR 15 and fH SCR 16 see ~~figure 6~~ Figure 17. AS can be seen in this model, heparin binding exists primarily at the ends – SCRs one and four. The middle of the molecule therefore does not contribute any to the binding (hence VCP SCR (2,3) showed no ability to bind).